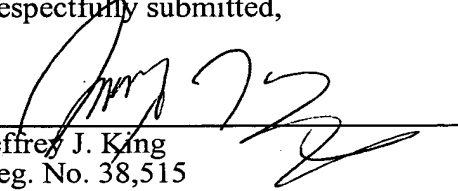


CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: May 29, 2001



Jeffrey J. King
Reg. No. 38,515

Customer No. 20350

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Phone (206) 467-9600
Fax (415) 576-0300
JJK:imp

SE 5008126 v1

APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph beginning at page 29, line 32, has been amended as follows:

Figure 6 illustrates construction of the PIV3-PIV2 chimeric antigenomic cDNA pFLC.PIV32hc (~~SEQ ID NO: xxx~~) encoding the full-length PIV2 HN and F proteins. The cDNA fragment containing the full-length PIV2 F ORF flanked by the indicated restriction sites (A1) was amplified from PIV2/V94 vRNA using RT-PCR and a PIV2 F specific primer pair (1, 2 in Table 9). This fragment was digested with NcoI plus BamHI (C1) and ligated to the NcoI-BamHI window of pLit.PIV31.fhc (B1) to generate pLit.PIV32Fhc (D1). In parallel, the cDNA fragment containing the full-length PIV2 HJN ORF flanked by the indicated restriction sites (A2) was amplified from PIV2/V94 vRNA using RT-PCR and a PIV2 HN specific primer pair (3, 4 in Table 9). This fragment was digested with NcoI plus HindIII (C2) and ligated to the NcoI-HindIII window of pLit.PIV31.HNhc (B2) to generate pLit.PIV32HNhc (D2). pLit.PIV32Fhc and pLit.PIV32HNhc were digested with PpuMI and SpeI and assembled together to generate pLit.PIV32hc (E). pLit.PIV32hc was further digested with BspEI and SpeI and introduced into the BspEI-SpeI window of p38'ΔPIV31hc (F) to generate p38'ΔPIC32hc (G). the chimeric PIV3-PIV2 construct was introduced into the BspEI-SphI window of pFLC.2G+hc to generate pFLC.PIC32hc (H).

The paragraph beginning at page 30, line 15, has been amended as follows:

Figure 7 depicts construction of full-length PIV3-PIV2 chimeric antigenomic cDNA pFLC.PIV32TM (~~SEQ ID NO: xx~~) and pFLC.PIV32TMcp45, which encode F and HN proteins containing PIV2-derived ectodomains and PIV3-derived transmembrane and cytoplasmic domains. The region of the PIV3 F ORF, in pLit.PIV3.F3a (A1), encoding the ectodomain was deleted (C1) by PCR using a PIV3 F specific primer pair (9, 10 in Table 9). The region of the PIV2 F ORF encoding the ectodomain was amplified from pLit.PIV32Fhc (B1) using PCR and PIV2 F specific primer pair (5, 6 in Table 9). The two resulting fragments (C1 and D1) were ligated to generate pLit.PIV32FTM (E1). In parallel, the region of the PIV3 HN ORF, in pLit.PIV3.HN4 (A2), encoding the ectodomain was deleted (C2) by PCR using a PIV3 HN specific primer pair (11, 12 in Table 9). The region of the PIV2 HN ORF encoding the

ectodomain was amplified from pLit.PIV32HNhc (B2) by PCR and a PIV2 HN specific primer pair (8, 9 in Table 9). Those two DNA fragments (C2 and D2) were ligated together to generate pLit.PIV32HNTM (E2). pLit.PIV32FTM and pLit.PIV32HNTM were digested with PpuMI and SpeI and assembled to generate pLit.PIV32TM (F). The BspEI-SpeI fragment from pLit.PIV32TM was ligated to the BspEI-SpeI window of p38'_PIV31hc (G) to generate p38'_PIV32TM (H). The insert containing chimeric PIV3-PIV2 F and HN was introduced as a 6.5 kb BspEI-SphI fragment into the BspEI-SphI window of pFLC.2G+.hc and pFLCcp45 to generate pFLC.PIV32TM and pFLC.PIV32TMcp45 (I), respectively.

The paragraph beginning at page 30, line 33, has been amended as follows:

Figure 8 shows construction of full-length PIV3-PIV2 chimeric antigenomic cDNA pFLC.PIV32CT (~~SEQ ID NO: XX~~) and pFLC.PIV32Ctcp45 which encode F and HN proteins containing a PIV2-derived ectodomain, a PIV2-derived transmembrane domain, and a PIV3-derived cytoplasmic domain. The region of the PIV3 F ORF in pLit.PIV3.F3a (A1) encoding the ectodomain and the transmembrane domain was deleted (C1) by PCR using a PIV3 F specific primer pair (17, 18 in Table 9). The region of the PIV2 F ORF encoding the ectodomain plus the transmembrane domain was amplified from pLit.PIV32Fhc (B1) using PCR and a PIV2 F specific primer pair (13, 14 in Table 9). The two resulting fragments (C1 and D1) were ligated to generate pLit.PIV32FCT (E1). In parallel, the region of the PIV3 HN ORF in pLit.PIV3.HN4 (A2), encoding the ectodomain and transmembrane domain was deleted (C2) by PCR using a PIV3 HN specific primer pair (19, 20 in Table 9). The region of the PIV2 HN ORF encoding the ectodomain plus the transmembrane domain was amplified from pLit.PIV32HNhc (B2) by PCR using a PIV2 HN specific primer pair (15, 16 in Table 9). Those two DNA fragments (C2 and D2) were ligated to generate pLit.PIV32HNCT (E2). pLit.PIV32FCT and pLit.PIV32HNCT were digested with PpuMI and SpeI and assembled to generate pLit.PIV32CT (F). The BspEI-SpeI fragment from pLit.PIV32CT was ligated to the BspEI-SpeI window of p38'_PIV31hc (G) to generate p38'_PIV32CT (H). The insert containing chimeric PIV3-PIV2 F and HN was introduced as a 6.5 kb BspEI-SphI fragment into the BspEI-SphI window of pFLC.2G+.hc and pFLC.cp45 to generate pFLC.PIV32CT and pFLC.PIV32CTcp45 (I), respectively.

The paragraph beginning at page 30, line 33, has been amended as follows:

-- Figure 9 details genetic structures of the PIV3-PIV2 chimeric viruses and the gene junction sequences for rPIV3-2CT and rPIV3-2TM. Panel A illustrates the genetic structures of rPIV3-2 chimeric viruses (middle three diagrams) are compared with that of rPIV3 (top diagram) and rPIV3-1 (bottom diagram) viruses. The *cp45* derivatives are shown marked with arrows depicting the relative positions of *cp45* mutations. For the *cp45* derivatives, only the F and HN genes are different while the remaining genes remained identical, all from PIV3. From top to bottom, the three chimeric PIV3-PIV2 viruses carry decreasing amount of PIV3 glycoprotein genes. Note that rPIV3-2, carrying the complete PIV2 HN and F ORF, was not recoverable. Panel B provides the nucleotide sequences of the junctions of the chimeric F and HN glycoprotein genes for rPIV3-2TM are given along with the protein translation. The shaded portions represent sequences from PIV2. The amino acids are numbered with respect to their positions in the corresponding wild type glycoproteins. Three extra nucleotides were inserted in PIV3-PIV2 HN TM as indicated to make the construct conform to rule of six. Panel C shows the nucleotide sequences of the junctions of the chimeric F and HN glycoprotein genes for rPIV3-2CT, given along with the protein translation. The shaded portions represent sequences from PIV2. The amino acids are numbered with respect to their positions in the corresponding wild type glycoproteins. GE= gene end; I= intergenic; GS= gene start; ORF= open reading frame; TM= transmembrane domain; CT= cytoplasmic domain; *= stop codon. The Figure includes SEQ ID NOs: 42-51.

The paragraph beginning at page 81, line 6, has been amended as follows:

The HA ORF of measles virus Edmonston strain was amplified from Edmonston wild type virus by reverse transcription polymerase chain reaction (RT-PCR). The nt sequence of the Edmonston wild type HA open reading frame (ORF) is in GenBank Accession # U03669, incorporated herein by reference (note that this sequence is the ORF only without the upstream 3 nts or the stop codon). Measles virus RNA was purified from clarified medium using TRIzol-LS (Life Technologies, Gaithersburg, MD) following the manufacturer's recommended procedure. RT-PCR was performed with the Advantage RT-for-PCR and Advantage-HF PCR kits (Clontech, Palo Alto, CA) following the recommended protocols. Primers were used to generate a PCR fragment spanning the entire ORF of the measles virus HA gene flanked by PIV3 non-coding sequence and *A*/II restriction sites. The forward primer 5'-
**TTAATCTTAAGAATATACAAATAAGAAAACTTAGGATTAAAGAGCGATGTCACCA
CAACGAGACCGGATAAATGCCTTCTAC-3'** (SEQ ID NO. 5) encodes an *A*/II site
(italicized) upstream of PIV3 noncoding sequence derived from the N/P gene junction-nts 3699-

3731(underlined), containing GE, IG and GS sequences (Figure 1A) and the beginning of the measles HA ORF (bolded) preceded by three non-HPIV3, non-measles virus nts designated in the primer. The reverse primer 5'-

**ATTATTGCTTAAGGTTTGTTCGGTGTCTGTTTCTTTGTTGGATCCTATCTGCGATTGGT
TCCATCTTC-3'** (SEQ ID NO. 6) encodes an *Afl*II site (italicized) downstream (in the positive-sense complement) of PIV3 noncoding sequence derived from the P gene, nt 3594-3623 (underlined), and the end of the measles HA ORF (bolded). The resultant PCR fragment was then digested with *Afl*II and cloned into p(*Afl*II N-P) and p(*Afl*II P-M) to create pUC119(HA N-P) and pUC119(HA P-M) respectively. pUC119(HA N-P) and pUC119(HA P-M) were sequenced over the entire *Afl*II insert using the dRhodamine Terminator Cycle Sequencing Ready Reaction (ABI prism, PE Applied Biosystems, Foster city, CA), and the sequence was confirmed to be correct.

The paragraph beginning at page 82, line 12, has been amended as follows:

A HPIV3 chimeric cDNA was constructed by PCR to include a heterologous polynucleotide sequence, exemplified by the measles virus HA gene, encoding a heterologous antigenic determinant of the measles virus, flanked by the transcription signals and the noncoding regions of the HPIV3 HN gene. This cDNA was designed to be combined with an rPIV3 vector as an extra gene following the HN gene. First, using Kunkel mutagenesis (Kunkel et al., Methods Enzymol. 154:367-382, 1987, incorporated herein by reference), a *Stu*I site was introduced in the 3'-noncoding region of the HN gene by mutating the AGACAA sequence at nts 8598-8603 of the antigenome to AGGCCT yielding plasmid p3/7(131)2G-Stu (Figure 1B). A cDNA containing the measles HA ORF flanked by HPIV3 sequences (see Figure 1B) was then constructed in three pieces by PCR. The first PCR synthesized the left-hand, upstream piece of the gene. The forward primer 5'-

**GACAATAGGCCTAAAAGGGAAATATAAAAACTTAGGAGTAAAGTTACGCAATCC-
3'** (SEQ ID NO. 7) contains a *Stu*I site (italicized) followed by HPIV3 sequence (underlined) which includes the downstream end of the HN gene (HPIV3 nts 8602-8620), an intergenic region, and the gene-start signal and sequence from the upstream end of the HN gene (HPIV3 nt 6733-6753). The reverse primer 5'-

**GTAGAACGCGTTTATCCGGTCTCGTTGTGGTGACATCTCGAATTTGGATTTGTCTA
TTGGGTCCTTCC-3'** (SEQ ID NO. 8) contains an *Mlu*I site (italicized) downstream of the start of the measles HA ORF (bolded) followed by the complement to HPIV3 nts 6744-6805 (underlined), which are part of the upstream HN noncoding region. The *Mlu*I site present in the

introduced measles virus ORF was created by changing nt 27 from T (in the wild type Edmonston HA gene) to C and nt 30 from C to G. Both of these changes are noncoding in the measles virus ORF. The PCR was performed using p3/7(131)2G-Stu as template. The resulting product, termed PCR fragment 1, is flanked by a *Stu*I site at the 5'-end and an *Mlu*I site at the 3'-end and contains the first 36 nt of the measles HA ORF downstream of noncoding sequence from the HPIV3 HN gene. The second PCR reaction synthesized the right-hand end of the HN gene. The forward primer 5'-

CAGTACCCCGGAAGATGGAACCAATCGCAGATAGTCATAATTAACCATAATATG
CATCAATCTATCTATAATACAA-3' (SEQ ID NO. 9) contains the *Xma*I (italics) and the end

of the measles HA ORF (bold), followed by HPIV3 nts 8525-8566 (underlined) representing part of the downstream nontranslated region of the HN gene. The reverse primer 5'-

CCATGTAATTGAATCCCCCAACACTAGC-3' (SEQ ID NO. 10), spans HPIV3 nts 11448-11475, located in the L gene. The template for the PCR was p3/7(131)2G-Stu. PCR fragment 2 which resulted from this reaction contains the last 35 nt of the measles HA ORF and approximately 2800 nt of the L ORF of PIV3 and is flanked by an *Xma*I site and an *Sph*I site (which occurs naturally at HPIV3 position 11317). The third PCR reaction amplified the largest, central portion of the measles HA ORF from the template cDNA pTM-7, a plasmid which contains the HA ORF of the Edmonston strain of measles virus supplied by the ATCC.

Sequence analysis of this plasmid showed that the measles virus HA ORF contained in PTM-7 contains 2 amino acid differences from pTM-7 ob the Edmonston wild type HA sequence used for insertion into the N-P and M-P junction, and these were at amino acid positions 46 (F to S) and at position 481 (Y to N). The forward primer 5'-

CGGATAAACGCGTTCTACAAAGATAACC-3' (SEQ ID NO. 11) (*Mlu*I site italicized) and reverse primer 5'-**CCATCTTCCCGGGTGACTGTGCAGC-3'** (SEQ ID NO. 12) (*Xma*I site italicized) amplified PCR fragment 3 which contained nts 19-1838 of the measles HA ORF. To

assemble the pieces, PCR fragment 1 was digested with *Stu*I and *Mlu*I while PCR fragment 3 was digested with *Mlu*I and *Xma*I. These two digested fragments were then cloned by triple ligation into the *Stu*I-*Xma*I window of pUC118 which had been modified to include a *Stu*I site in its multiple cloning region. The resultant plasmid, pUC118(HA 1+3) was digested with *Stu*I and *Xma*I while PCR fragment 2 was digested with *Xma*I and *Sph*I. The two digested products were then cloned into the *Stu*I-*Sph*I window of p3/7(131)2G-Stu, resulting in the plasmid pFLC(HA HN-L). The *Stu*I-*Sph*I fragment, including the entire measles HA ORF, was then sequenced using the dRhodamine Terminator Cycle Sequencing Ready Reaction (ABI prism, PE Applied Biosystems, Foster city, CA). The chimeric construct sequence was confirmed. In this way, the

measles virus HA ORF flanked by HPIV3 transcription signals was inserted as an extra gene into the N/P, P/M, or HN/L junction of an antigenomic cDNA vector comprising a wild type HPIV3 or into the N/P or P/M junction of an antigenomic cDNA vector comprising an attenuated HPIV3.

The paragraph beginning at page 95, line 5, has been amended as follows:

rPIV3-1 is a recombinant chimeric HPIV3 in which the HN and F genes have been replaced by those of HPIV1 (see, e.g., Skiadopoulos et al., Vaccine 18:503-510, 1999; Tao et al., Vaccine 17:1100-1108, 1999; U.S. Patent Application Serial No. 09/083,793, filed May 22, 1998, each incorporated herein by reference). In the present example, the HN gene of HPIV2 was inserted into the rPIV3-1 chimeric virus that served as a vector to produce a chimeric derivative virus, bearing an introduced heterologous antigenic determinant from HPIV2, able to protect against both HPIV1 and HPIV2. The HPIV2 HN gene also was inserted into an attenuated derivative of rPIV3-1, designated rPIV3-1_{cp45}, which contains 12 of the 15 *cp45* mutations, i.e., those mutations on genes other than HN and F, inserted into the rPIV3 backbone (Skiadopoulos et al., Vaccine 18:503-510, 1999). The source of the HPIV2 wild type virus was the wild type strain V9412-6 (designated PIV2/V94) (Tao et al., Vaccine 17:1100-1108, 1999), which was isolated in Vero cells from a nasal wash that was obtained in 1994 from a child with a natural HPIV2 infection. PIV2/V94 was plaque purified 3 times on Vero cells before being amplified twice on Vero cells using OptiMEM tissue culture medium without FBS. A cDNA clone of the HN gene of PIV2/V94 was generated from virion RNA by reverse transcription (RT) using random hexamers and Superscript Preamplification System (Life Technologies) followed by PCR using Advantage cDNA Synthesis kit (Clontech, Palo Alto, CA) and synthetic primers which introduced *NcoI*-*HindIII* sites flanking the HN cDNA (Figure 3A). The sequences of these primers were: (with HPIV specific sequences in upper case, restriction sites underlined, nts which are non-HPIV or which are altered from wt in lower case, and start and stop codons in bold), upstream HPIV2 HN 5'-gggcc**ATG**GGAAGATTACAGCAAT-3' (SEQ ID NO. 13); downstream HPIV2 HN 5'-caataagc**TTA**AAGCATTAGTTCCC-3' (SEQ ID NO. 14). The HN PCR fragment was digested with *NcoI*-*HindIII* and cloned into pLit.PIV31HNhc to generate pLit.32HNhc (Figure 3 B). The HPIV2 HN heterologous gene insert in pLit.32HNhc was completely sequenced using the ThermoSequenase Kit and ³³P-labeled terminators (Pharmacia Amersham, Piscataway, NJ) and was confirmed to contain the authentic sequence of the PIV2/94 HN coding region.

The paragraph beginning at page 96, line 1, has been amended as follows:

The HPIV2 HN gene in pLit.32HNhc was further modified by PCR and Deep Vent thermostable DNA polymerase (New England Biolab, Beverly, MA) to introduce *Ppu*MI sites for cloning into the unique *Ppu*MI site in p38'ΔPIV31hc, Figure 3C (Skiadopoulos et al., Vaccine 18:503-510, 1999). The sequences of these primers were (with HPIV specific sequences in upper case, relevant restriction sites underlined, non-HPIV nt or nt altered from wt in lower case): upstream HPIV2 HN

5'-gcatgggcccGAGGAAGGACCCAATAGACA-3' (SEQ ID NO. 15); downstream HPIV2 HN 5'-cccgggtcctgATTTC^{CG}GAGCACGCTTTG-3' (SEQ ID NO. 16). The modified cDNA bearing the HPIV2 HN ORF consists of (from left to right) a partial 5'-untranslated region (5'-UTR) of HPIV3 HN including the *Ppu*MI site at the 5'-end, the HPIV2 HN ORF, the 3'-UTR of HPIV3 HN, a complete set of HPIV3 transcription signals (i.e. gene stop, intergenic region and gene start sequences) whose sequences match those at the HPIV3 HN and L gene junction, a partial 5'-UTR of HPIV3 L, and an added *Ppu*MI site at its 3'-end (Figure 3C). This fragment was digested with *Ppu*MI and inserted into p38'ΔPIV31hc digested with *Ppu*MI to generate p38'ΔPIV31hc.2HN (Figure 3D). The inserted *Ppu*MI cassette was sequenced in full and found to be as designed. The insert from p38'ΔPIV31hc.2HN was isolated as a 8.5 kb BspEI-SphI fragment and introduced into the BspEI-SphI window of pFLC.2G+.hc or pFLC*cp*45 to generate pFLC.31hc.2HN or pFLC.31hc.*cp*45.2HN, respectively (Figure 3, E and F). pFLC.2G+.hc and pFLC*cp*45 are full-length antigenomic clones encoding wt rPIV3-1 and rPIV3*cp*45, respectively, as described previously (Skiadopoulos et al., J. Virol. 73:1374-81, 1999; Tao et al., J. Virol. 72:2955-2961, 1998, each incorporated herein by reference).

The paragraph beginning at page 97, line 1, has been amended as follows:

To determine if the rPIV3-1.2HN and rPIV3-1*cp*45.2HN recombinants contain the heterologous HPIV2 HN gene, viral RNA from each recovered recombinant chimeric virus was amplified on LLC-MK2 cells and concentrated by polyethylene glycol (PEG) precipitation (Mbiguino et al., J. Virol. Methods 31:161-170, 1991, incorporated herein by reference). Virion RNA (vRNA) was extracted with Trizol (Life Technologies) and used as template to synthesize first strand cDNA using Superscript Preamplification system (Life Technologies, Gaithersburg, MD) and random hexamer primers as described above. The synthesized cDNA was amplified by PCR with the Advantage cDNA Synthesis kit (Clontech, Palo Alto, CA) with primers specific for HPIV1 F and HPIV1 HN coding region (for HPIV1 F 5'-

AGTGGCTAATTGCATTGCATCCACAT-3' (SEQ ID NO. 17) and for HPIV1 HN 5'-GCCGTCTGCATGGTGAATAGCAAT-3' (SEQ ID NO. 18). The relative locations of the PIV1 F and HN primers are indicated by arrows in Figures 3 and 4. Amplified DNA fragments were digested and analyzed on agarose gels (Figure 4). Data for rPIV3-1 $cp45.2HN$ is not shown, but was comparable and confirmed in structure. rPIV3-1.2HN and rPIV3-1 $cp45.2HN$ each contained the insert of the expected size, and the digestion patterns with a number of restriction enzymes confirmed the identity and authenticity of the inserts. The presence of the $cp45$ mutations in rPIV3-1 $cp45.2HN$ was also confirmed.

The paragraph beginning at page 106, line 18, has been amended as follows:

A DNA encoding a full-length PIV3 antigenomic RNA was constructed in which the PIV3 F and HN ORFs were replaced by their PIV2 counterparts following the strategy described previously (Tao et al., J. Virol. 72:2955-2961, 1998) for PIV3-PIV1. Details of this construction are presented in Figure 6. PIV2/V94 propagated in Vero cells was concentrated and virion RNA (vRNA) was extracted from the virus pellet using Trizol reagent. The F and HN ORFs of PIV2/V94 were reverse transcribed from vRNA using random hexamer primers and the SuperScript Preamplification System before being amplified by PCR using the cDNA Advantage kit and primer pairs specific to PIV2 F and HN genes, respectively (1, 2 and 3, 4; Table 9). The amplified cDNA fragment of PIV2 F ORF was digested with NcoI plus BamHI and ligated into the NcoI-BamHI window of pLit.PIV31.Fhc (Tao et al., J. Virol. 72:2955-2961, 1998, incorporated herein by reference) to generate pLit.PIV32Fhc. The BspEI site in the PIV3 full-length cDNA is unique and we planned to use it to exchange segments between cDNAs (see Figures 6-8). Therefore, a BspEI site that was found in the PIV2 F ORF was removed by site-directed mutagenesis without affecting the amino acid sequence. The cDNA fragment of PIV2 HN ORF was digested with NcoI plus HindIII and ligated into the NcoI-HindIII window of pLit.PIV31.HNhc (Tao et al., J. Virol. 72:2955-2961, 1998) to generate pLit.PIV32HNhc. The PIV2 ORFs in pLit.PIV32Fhc and pLit.PIV32HNhc were sequenced, and the sequence was found to be as designed. The nucleotide sequences for the PIV2 F and HN ORFs are submitted in the GenBank (Accession No. pending). pLit.PIV32Fhc and pLit.PIV32HNhc were each digested with PpuMI plus SpeI and assembled to generate pLit.PIV32hc. The 4 kb BspEI-SpeI fragment of pLit.PIV32hc was introduced into the BspEI-SpeI window of p38' Δ PIV31hc (Skiadopoulos et al., Vaccine 18:503-510, 1999, incorporated herein by reference) to generate p38' Δ PIV32hc. The 6.5 kb fragment, generated by BspEI and SphI digestion of p38' Δ PIV32hc,

containing the PIV2 full-length F and HN ORFs was introduced into the BspEI-SphI window of pFLC.2G+.hc (Tao et al., J. Virol. 72:2955-2961, 1998) to generate pFLC.PIV32hc (Figure 6; Table 10 = SEQ ID NO: 19).

The heading of Table 10, beginning at page 110, has been amended as follows:

Table 10 (SEQ ID NO: 19)

Table 9 has been amended only by the addition of Sequence Identification Numbers. A marked up version of this new Table 9 is attached.

Appendix

Marked-up Version of Table 9

Table 9. Primers used in construction of PIV3-2 full-length chimeric antigenomic cDNAs

Primer No.	Gene	Direction	Position		Used in the construction or characterization of:	Sequence ^a
			Beginning	End		
1	PIV2 F	sense	PIV2 F start codon 5070 ^b	20 bp down stream 5091	pFLC.PIV32hc	gtaccATGgATCACCTGCATCCAAT (SEQ ID NO. 20)
2	PIV2 F	antisense	PIV2 F stop codon 6732 ^b	20 bp upstream 6705 ^b	pFLC.PIV32hc	tgtagatccTAAGATATCCCATATATGTTTC (SEQ ID NO. 21)
3	PIV2 HN	sense	PIV2 HN start codon 6837 ^b	18 bp down stream 6856 ^b	pFLC.PIV32hc	gggccATGGAAgATTACAGCAAT (SEQ ID NO. 13)
4	PIV2 HN	antisense	PIV2 HN stop codon 8558 ^b	17 bp upstream 8538 ^b	pFLC.PIV32hc	caataagcTTAAAGCATTAGTTCC (SEQ ID NO. 14)
5	PIV2 F	sense	5069 ^c	5088 ^c	pFLC.PIV32TM	ATGCATCACCTGCATCCAAT (SEQ ID NO. 22)
6	PIV2 F	antisense	6538 ^c	6517 ^c	pFLC.PIV32TM	TAGTGAATAAAAGTGTCTTGGCT (SEQ ID NO. 23)
7	PIV2 HN	sense	6962 ^c	6985 ^c	pFLC.PIV32TM	CATGAGATAATTTCATCTTGATGTT (SEQ ID NO. 24)
8	PIV2 HN	antisense	8560 ^c	8537 ^c	pFLC.PIV32TM	agcTTAAAGCATTAGTTCCTTAA (SEQ ID NO. 25)
9	PIV3 F	sense	6539 ^c	6566 ^c	pFLC.PIV32TM	ATCATAATTATTTTGATAATGATCATTA (SEQ ID NO. 26)
10	PIV3 F	antisense	5068 ^c	5050 ^c	pFLC.PIV32TM	GTTCAGTGTCTTGTGTGTT (SEQ ID NO. 27)
11	PIV3 HN	sense	8561 ^c	8587 ^c	pFLC.PIV32TM	TCATAATTAAACCATAAATATGCATCAAT (SEQ ID NO. 28)
12	PIV3 HN	antisense	6961 ^c	6938 ^c	pFLC.PIV32TM	GATGGAATTAAATTAGCACTATGAT (SEQ ID NO. 29)
13	PIV2 F	sense	5069 ^d	5088 ^d	pFLC.PIV32CT	ATGCATCACCTGCATCCAAT

Appendix
Marked-up Version of Table 9

14	PIV2 F	antisense	6607 ^d	6589 ^d	pFLC.PIV32CT	<u>(SEQ ID NO. 30)</u> GATGATGTAGGCAATCAGC <u>(SEQ ID NO. 31)</u>
15	PIV2 HN	sense	6887 ^d	6904 ^d	pFLC.PIV32CT	ACTGCCACAAATTCCTTGGC <u>(SEQ ID NO. 32)</u>
16	PIV2 HN	antisense	8536 ^d	8511 ^d	pFLC.PIV32CT	TTAAAGCAATTAGTTCCTTAAAAATG <u>(SEQ ID NO. 33)</u>
17	PIV3 F	sense	6620 ^d	6642 ^d	pFLC.PIV32CT	AAGTATTACAGAAATTCAAAAGAG <u>(SEQ ID NO. 34)</u>
18	PIV3 F	antisense	5068 ^d	5050 ^d	pFLC.PIV32CT	GTTCAGTGCTTGTGTGT <u>(SEQ ID NO. 27)</u>
19	PIV3 HN	sense	8525 ^d	8551 ^d	pFLC.PIV32CT	TCATAATTAACCATATAATGCATCAAT <u>(SEQ ID NO. 28)</u>
20	PIV3 HN	antisense	6898 ^d	6879 ^d	pFLC.PIV32CT	CTTATTAGTGAGCTTGTGTG <u>(SEQ ID NO. 35)</u>
21	PIV2 F	Sense	6608 ^{c,d}	6630 ^{c,d}	Chimera confirmation	ACCGCAGCTGTAGCAATAGT <u>(SEQ ID NO. 36)</u>
22	PIV2 HN	antisense	7522 ^c 7501 ^d	7502 ^c 7481 ^d	Chimera confirmation	GATTCCATCACTTAGGTAAAT <u>(SEQ ID NO. 37)</u>
23	PIV3 M	sense	4759 ^{c,d}	4780 ^{c,d}	Chimera confirmation	GATACTATCCTAATATTATGC <u>(SEQ ID NO. 38)</u>
24	PIV3 L	antisense	9100 ^c 9076 ^d	9081 ^c 9057 ^d	Chimera confirmation	GCTAATTTTGATAGCACATT <u>(SEQ ID NO. 39)</u>

^a All the primers are annotated in that the PIV specific sequences are in uppercase, non-PIV sequences in lowercase, start and stop codons in bold, and restriction sites underlined.

^b The numbers are the nt positions in the full-length antigenomic cDNA construct pFLC.PIV32hc.

^c The numbers are the nt positions in the full-length antigenomic cDNA construct pFLC.PIV32TM and pFLC.PIV32TMcp45.

^d The numbers are the nt positions in the full-length antigenomic cDNA construct pFLC.PIV32CT and pFLC.PIV32CTcp45.

The paragraph beginning at page 115, line 1, has been amended as follows:

--In a second strategy (Figure 7), chimeric PIV3-PIV2 F and HN ORFs rather than the complete ORF exchange were constructed in which regions of the PIV2 F and HN ORFs encoding the ectodomains were amplified from pLit.PIV32Fhc and pLit.PIV32HNhc, respectively, using PCR, Vent DNA polymerase (NEB, Beverly, MA), and primer pairs specific to PIV2 F (5, 6 in Table 9) and HN (7, 8 in Table 9). In parallel, the regions of PIV3 F and HN ORFs encoding the ectodomains were deleted from their cDNA subclones pLit.PIV3.F3a and pLit.PIV3.HN4 (Tao et al., *J. Virol.* 72:2955-2961, 1998, incorporated herein by reference), respectively, using PCR, Vent DNA polymerase, and primer pairs specific to PIV3 F (9, 10 in Table 9) and HN (11, 12 in Table 9). The amplified F and HN cDNA fragments of PIV2 and PIV3 were purified from agarose gels and ligated to generate pLit.PIV32FTM and pLit.PIV32HNTM, respectively. The chimeric F and HN constructs were digested with PpuMI plus SpeI and assembled together to generate pLit.PIV32TM, which was subsequently sequenced with the dRhodamine dye terminator sequencing kit across its PIV specific region in its entirety and found to be as designed. The 4 kb BspEI-SpeI fragment from pLit.PIV32TM was then introduced into the BspEI-SpeI window of p38' Δ PIV31hc to generate p38' Δ PIV32TM. The 6.5 kb BspEI-SphI fragment from p38' Δ PIV32TM, containing the PIV3-PIV2 chimeric F and HN genes, was introduced into the BspEI-SphI window of pFLC.2G+.hc and pFLCcp45 (Skiadopoulos et al., *J. Virol.* 73:1374-81, 1999, incorporated herein by reference) to generate pFLC.PIV32TM (Table 11; SEQ ID NO: 40) and pFLC.PIV32TMcp45, respectively. The nucleotide sequence of the BspEI-SpeI fragment, containing the chimeric PIV3-PIV2 F and HN genes, is submitted in the GenBank (Accession No. pending).

The heading of Table 11, beginning at page 116, has been amended as follows:

TABLE 11 (SEQ ID NO. 40)

The paragraph beginning at page 121, line 1, has been amended as follows:

In a third strategy (Figure 8), chimeric PIV3-PIV2 F and HN genes were constructed in which regions of the PIV2 F and HN ORFs encoding the ectodomains and the transmembrane domains were amplified from pLit.PIV32Fhc and pLit.PIV32HNhc, respectively, using PCR, Vent DNA polymerase, and primer pairs specific to PIV2 F (13, 14 in Table 9) and PIV2 HN (15, 16 in Table 9). In parallel, the partial ORFs of PIV3 F and HN genes encoding the

ectodomains plus transmembrane domains were deleted from their cDNA subclones pLit.PIV3.F3a and pLit.PIV3.HN4 (Tao et al., J. Virol. 72:2955-2961, 1998, incorporated herein by reference), respectively, using PCR, Vent DNA polymerase, and primer pairs specific to PIV3 F (17, 18 in Table 1) and PIV3 HN (19, 20 in Table 9). The F and HN cDNA fragments of PIV2 and PIV3 were gel purified and ligated to generate pLit.PIV32FCT and pLit.PIV32HNCT, respectively. The chimeric F and HN constructs were digested with PpuMI plus SpeI and assembled together to generate pLit.PIV32CT, which was sequenced across the PIV specific region in its entirety and found to be as designed. The 4 kb BspEI-SpeI fragment from pLit.PIV32CT was introduced into the BspEI-SpeI window of p38' Δ PIV31hc to generate p38' Δ PIV32CT. The 6.5 kb BspEI-SphI fragment from p38' Δ PIV32CT, containing the PIV3-PIV2 F and HN chimeric genes, was introduced into the BspEI-SphI window of pFLC.2G+.hc and pFLCcp45, to generate pFLC.PIV32CT (Table 12, SEQ ID NO: 41) and pFLC.PIV32CTcp45, respectively. The nucleotide sequence of this *BspEI-SpeI* fragment is submitted in the GenBank (Accession No. pending).

The heading of Table 12, beginning at page 122, has been amended as follows:

TABLE 12 (SEQ ID NO. 41)